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Title: Structural brain network analysis in families multiply affected with bipolar I disorder

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Abstract

Disrupted structural connectivity is associated with psychiatric illnesses including bipolar disorder (BP). Here we utilise structural brain network analysis to investigate connectivity abnormalities in multiply affected BP I families, to assess the utility of dysconnectivity as a biomarker and its' endophenotypic potential. Magnetic Resonance Diffusion images for 19 BP type I patients in remission, 21 of their first degree unaffected relatives and 18 unrelated healthy controls underwent tractography. Using the automated anatomical labelling atlas to define nodes, a connectivity matrix was generated for each subject. Network metrics were extracted with the Brain Connectivity Toolbox then analysed for group differences, accounting for potential confounding effects of age, gender and familial association. Whole brain analysis revealed no differences between groups. Analysis of specific mainly frontal regions, previously implicated as potentially endophenotypic by fMRI analysis of the same cohort, revealed a significant effect of group in the right medial superior frontal gyrus and left middle frontal gyrus driven by reduced organisation in patients compared to controls. The organisation of whole brain networks of those affected with BP I does not differ from their unaffected relatives or healthy controls. However in discrete frontal regions, anatomical connectivity is disrupted in patients but not their unaffected relatives.

Key words: Familial Bipolar disorder, Network analysis, Diffusion weighted MRI, Endophenotype

1. Introduction

The brain is an immensely complex system which is both highly specialised and integrated. Through recent advances in diffusion weighted Magnetic Resonance Imaging (MRI) and the application of graph theory we can now model anatomical connectivity within the brain as a network. To date multiple studies have used network analysis to investigate the organisation of the brain, determining it to be a vastly well organised network displaying small world properties and a large degree of clustering, where communities of grey matter structures are more highly connected to each other than to regions in other clusters (Hagmann et al., 2007, 2008; Iturria-Medina et al., 2008; Gong et al., 2009; Bassett et al., 2011). This technique has also been successfully implemented in a few studies to examine anatomical network abnormalities in disease (Lo et al., 2010; van den Heuvel et al., 2010; Caeyenberghs et al., 2012, 2014; Leow et al., 2013; Reijmer et al., 2013a, 2013b). Most relevant of the network analysis literature for the current study is an investigation of bipolar disorder (BP) which revealed impaired connectivity between hemispheres for the BP patients compared to controls (Leow et al., 2013). Considering the recent consensus review of BP that determined two key emotional control networks are dysfunctional in BP (Strakowski et al., 2012) we determined to utilise network analysis to evaluate the structural networks of the brain and determine at a network rather than local level what is abnormal in BP. The rationale for this form of investigation has been further strengthened by the findings of Wessa and colleagues (2014) whose review continued to develop neurobiological models of BP which attribute BP of abnormalities in neural networks; including networks involving the amygdala, prefrontal cortex and anterior cingulate gyrus. Here we utilise network analysis techniques to investigate differences in structural connectivity in BP I patients, their first degree relatives and healthy controls, in order to further assess dysconnectivity between grey matter regions as a biomarker in BP and as a potential endophenotypic marker. This is the first attempt to do so in BP. Metrics derived from diffusion imaging have previously been shown to be highly heritability (Kochunov et al., 2010; Geng et al., 2012; Jahanshad et al., 2013) and thus have potential as endophenotypic markers for psychiatric disorders. Though these findings are not

network based it is reasonable to assume the heritability extends to network measures also. These data have previously been analysed using Tract-based spatial statistics (TBSS) and tractography, two complimentary methods to investigate focal abnormalities (Chaddock et al., 2009; Emsell et al., 2013), whereas the novel approach used herein concerns itself with the network organisation of the brain rather than local abnormalities.

There is a vast array of network metrics available for investigation; herein we restricted our analysis to the following robust and commonly used metrics: clustering coefficient, global and local efficiency and characteristic path length. These, and many others, have been described in detail by Rubinov and Sporns (2010) but below is a brief introduction to network analysis and description of each.

The network is a mathematical model of how the brain is organised; it is comprised of nodes and edges. Nodes in this case are distinct anatomical grey matter areas whereas the edges are the white matter connections between them derived using diffusion weighted MRI and tractography. Anatomical networks are simultaneously both highly segregated and integrated. In this study we investigate local and global measures of each.

Characteristic path length (CPL) is a global measure of integration within a network. The shortest path length is the fewest number of edges that must be travelled to go from one node to another (Bullmore and Sporns, 2009) and CPL is the average shortest path length between each pair of nodes in the network. Global efficiency (E_g) is related, as it is the average inverse of the shortest path length. These differ in that CPL is primarily affected by long paths while the E_g as the inverse is primarily influenced by short paths. Local efficiency (E_l) is, as the name suggests, a local measure of efficiency or integration. Clustering coefficient (CC) is a measure of segregation within the network. It is the fraction of nodes' neighbours that are also neighbours of each other; it also quantifies the number of connections between the nearest neighbours of a node as a proportion of the maximum number of possible connections. Higher CC indicates higher segregation and clustering around that

node. The CC for the whole brain is the average prevalence of clustered connectivity around individual nodes (Rubinov and Sporns, 2010).

Below we use network analysis to test the hypothesis that brain structural connectivity is disrupted in patients with BP and investigate the potential of network analysis measures as endophenotypic markers of BP by including unaffected first degree relatives in our analysis.

2. Methodology

2.1 Participants

The majority of participants had previously taken part in structural (McDonald et al., 2004, 2005) and functional studies of bipolar disorder (Drapier et al., 2008; Allin et al., 2010; Surguladze et al., 2010; Radua et al., 2013). All participated in our previous diffusion studies employing voxel based analysis (Chaddock et al., 2009) and tractography (Emsell et al., 2013). Subject demographics have been described in detail elsewhere (Emsell et al., 2013) and are summarised in table 1. Nineteen BP I patients in remission, 21 of their first degree relatives (4 parents, 10 siblings and 7 children) and 18 unrelated healthy volunteers took part in this study after giving written informed consent. Unaffected relatives did not fulfill criteria for bipolar disorder or psychotic disorder but other nonpsychotic lifetime diagnoses were not exclusion criteria for either relative or control groups. Three participants from the relatives group had a lifetime diagnosis of Major Depressive disorder (MDD), 1 had Substance Abuse disorder and 1 had Generalized Anxiety disorder. One relative was taking cipramil for the 2 months prior to scanning for their MDD. The study was approved by the London – Camberwell St Giles National Research Ethics Service Committee (formerly the Joint South London and Maudsley and the Institute of Psychiatry Research Ethics Committee).

Table 1 Subject demographics

	Bipolar I (19)	Relatives (21)	Controls (18)	Test statistic	<i>p</i> - value
Age (years), mean (SD)	43.26 (10.16)	42.52 (13.65)	41.72 (12.24)	$F_{(2)} = 0.07$	0.93
Age (years), range	30-62	21-64	26-63		
Gender, m/f	9/10	12/9	10/8	$\chi^2 = 0.45$	0.80
Full-scale IQ, mean (SD)	114.6 (15.4)	118.8 (7.5)	114.9 (13.9)	$F_{(2,53)} = 1.02$	0.47
Parental SES ^a	9	13	11	$\chi^2 = 1.03$	0.60
BDI, mean (SD)	7.9 (7.0)	5.0 (3.5)	3.4 (3.7)	$F_{(2,51)} = 3.49$	0.038
ASRM, mean (SD)	3.5 (2.6)	1.8 (2.5)	1.0 (1.8)	$F_{(2,51)} = 4.95$	0.011

Age symptom onset (years) ^b , mean (SD)	22.94 (5.67)	
Duration of illness (years) ^b , mean (SD)	20.25 (10.84)	
Depressive episodes ^b , mean (SD)	5.9 (6.3)	
Manic episodes ^b , mean (SD)	7.0 (7.01)	
Hospitalisations ^b , mean (SD)	3.3 (1.4)	
Current psychotropic medication ^b , n		
None	3	
Lithium	9	
Mood Stabilizers, other(e.g.Valproate)	7	
Antidepressants	4	1
Antipsychotics	4	

Table 1 legend ASRM – Altman Self-Rating Mania scale, BDI – Beck Depression Inventory, m – male, f – female, SD – standard deviation, SES – socio-economic status. ^aClass I or II (Professional, managerial and technical occupations). Based on details of parental occupation at time of participants birth. ^bData based on n=16 BP patients, data unavailable for the remaining 3.

2.2 Acquisition

Diffusion-weighted data were acquired using a GE Signa 1.5T LX MRI system (General Electric, Milwaukee, Wisconsin, USA) with an echo planar imaging sequence, peripherally gated to the cardiac cycle. Seven non-diffusion weighted reference images (B_0) and 64 images with a diffusion gradient ($b = 1300 \text{ s/mm}^2$) were acquired at each of 60 axial slices. This gave a 2.5 mm^3 voxel dimension which was reconstructed to a $1.875 \times 1.875 \text{ mm}$ in-plane pixel size.

2.3 Pre-processing

Correction of images for eddy current induced geometric distortions and subject motion, including adjustment of the B-matrix (Leemans and Jones, 2009), was performed with the diffusion MRI toolbox ExploreDTI v4.8.3 (Leemans et al., 2009). Tensor estimation was performed using robust estimation of tensors by outlier rejection, RESTORE, (Chang et al., 2005). Data quality was determined visually, using steps outlined previously (Tournier et al., 2011). Two data sets were omitted from analysis due to artefacts, one from the relative group and one from the patient group,

this did not affect the age or gender balance between groups (age: $F_{(2)} = 0.18$, $p=0.83$, gender: $\chi^2 = 0.80$, $p = 0.67$).

2.5 Whole Brain Tractography

Whole brain tractography using constrained spherical deconvolution (CSD, Tournier et al., 2007) was performed with ExploreDTI v4.8.3 (Leemans et al., 2009; Jeurissen et al., 2011). The following parameters were applied: maximum spherical harmonic order for CSD 8, seed point resolution 2 mm³, step size 1 mm, angle threshold 30°, fibre orientation distribution threshold 0.1 and fibre length range 50-500 mm.

2.6 Connectivity matrix

A weighted connectivity matrix for each subject was generated in ExploreDTI using the AAL atlas (Tzourio-Mazoyer et al., 2002) regions as nodes. The cerebellum was excluded, leaving a total of 90 nodes, of which 78 were cortical and 12 sub-cortical. The matrices used were weighted by number of streamlines connecting node i to node j . An overview of the processing pipeline can be seen in Fig. 1.

Insert Fig. 1 here

Fig. 1 Pipeline of image processing

Fig. 1 legend (a) Fractional anisotropy image; (b) whole brain fibre tractography. (c) Shows the AAL atlas used to determine nodes. (d) Shows a connectivity graph generated with ExploreDTI, nodes from the AAL atlas are in red and the edges between nodes are shown in green, a semi opaque left hemisphere has been included for anatomical reference.

2.7 Brain Connectivity Toolbox (BCT)

The Brain Connectivity Toolbox (Rubinov and Sporns, 2010) was used to generate the following connectivity parameters: clustering coefficient, characteristic path length, local and global efficiency, all of which are described in detail by Rubinov and Sporns (2010). These were analysed in our primary analysis for the whole brain network. A further analysis was completed on a narrower range of nodes implicated in the pathophysiology of BP in this cohort by virtue of abnormal functioning identified through our previous fMRI studies using cognitive and emotional stimuli, namely left amygdala, left putamen, left and right frontal superior medial nodes (Surguladze et al., 2010); a left frontal cluster of nodes; middle, middle orbital, inferior triangularis and inferior orbital (Drapier et al., 2008); left and right precuneus, left precentral, left rolandic operculum and left frontal medial orbital (Allin et al., 2010). The clustering coefficient and local efficiency of these selected nodes were extracted from the matrices and analysed. They are highlighted in green in Fig. 2. This was an exploratory analysis and we therefore used a data-driven approach to select a small number of the potential nodes that could have been investigated had we used all those previously implicated in the literature. We focussed on regions identified as abnormal in prior functional studies from this cohort as we considered these may be associated with abnormal structural connectivity. Furthermore, our selection does include some of the most commonly implicated regions in the literature, such as the amygdala and prefrontal regions (Strakowski et al., 2012; Wessa et al., 2014).

Insert Fig. 2 here

Fig. 2 Nodes from AAL atlas used for analysis

Fig. 2 legend Figure displays all nodes used in analysis, green indicates the nodes chosen for further node specific analysis, based upon prior abnormalities identified through functional imaging studies. Nodes were defined based on regions from the automated anatomical labelling (AAL) atlas. The image was generated with ExploreDTI. The figure displays an axial view of the brain from above using the neurological convention; therefore left side of the image corresponds to the left hemisphere and likewise for the right.

2.8 Statistical analysis

Age and gender balance between groups were determined by 1-way ANOVA and Chi Squared statistical tests respectively in the PASW18 statistical program.

Regression analysis in STATA version 8 was used to determine if group could predict connectivity metrics (clustering coefficient, characteristic path length, global and local efficiency) (xi:regress and test commands). We accounted for the inclusion of related individuals by using STATA's "cluster" command for linear regression, which specifies that observations are independent across families but not within families. Effects of age and gender were also covaried for. Post-hoc pair-wise comparisons of groups were carried out in a similar fashion (xi:regress command) where an overall significant effect as defined by $p \leq 0.05$ was detected in the overall groups comparison. This was carried out for the whole brain and specific nodes previously implicated in disrupted functional connectivity using fMRI in this sample (see section 2.7 and Fig. 1). Results were left uncorrected for multiple comparisons in this exploratory analysis.

2.9 Correlation analysis

Measures from nodes found to be significantly different were investigated for their relation to clinical measures within the BP group; age of illness onset, duration of illness and symptom severity.

3. Results

3.1 Demographics

Groups were balanced for age and gender (Table 1), both before and following the removal of the two corrupted image sets noted above (age: $F_{(2)} = 0.18$, $p=0.83$, gender: $\chi^2 = 0.80$, $p = 0.67$).

3.2 BCT Whole Brain Network

Primary analysis of the whole brain network revealed no differences between groups (CPL: $F=0.43$, $p=0.66$; E_g : $F=1.66$, $p=0.20$; E_i : $F=2.08$, $p=0.14$; CC: $F=2.25$, $p=0.12$).

3.3 Node Specific

Node specific analysis revealed a significant but small ($R^2 = 13\text{-}20\%$) effect of group on clustering coefficient and local efficiency in the frontal superior medial right and middle left nodes (Table 2).

Table 2 Mean (SD) node specific network analysis metrics in each participant group

Node		Bipolar 1	Relatives	Controls	Group Comparison	
<u>Clustering Coefficient</u>					Test-statistic	<i>p</i> -value
Frontal-	superior medial left	35.86 (8.37)	43.64 (14.19)	43.27 (11.32)	3.02	0.06
	superior medial right	32.61 (6.72)	38.67 (13.56)	40.86 (10.62)	5.18	0.01*
	middle left	31.57 (10.35)	39.07 (14.56)	40.50 (10.72)	3.17	0.05*
	middle orbital left	22.41 (8.54)	23.81 (9.48)	22.88 (7.51)	0.06	0.94
	inferior triangularis left	45.94 (10.79)	52.85 (15.57)	50.48 (17.70)	1.38	0.26
	inferior orbital left	20.28 (5.86)	19.91 (5.64)	20.82 (5.63)	0.15	0.86
	medial orbital left	38.91 (11.85)	46.75 (14.15)	40.56 (1.18)	1.88	0.17
Amygdala left		21.43 (5.93)	21.84 (7.37)	21.97 (4.32)	0.02	0.98
Putamen left		43.51 (8.06)	45.61 (6.50)	46.19 (8.97)	0.76	0.48
Rolandic operculum left		31.91 (8.38)	36.86 (12.05)	36.41 (9.16)	1.23	0.30
Precuneus left		52.74 (10.37)	56.16 (11.22)	55.81 (15.60)	0.32	0.73
Precuneus right		58.90 (9.60)	63.26 (11.47)	65.10 (14.81)	1.45	0.25
Precentral left		54.78 (13.84)	61.36 (25.98)	56.38 (15.98)	0.88	0.42
<u>Local efficiency</u>						
Frontal-	superior medial left	60.02 (13.34)	71.23 (21.66)	70.14 (15.85)	2.75	0.08
	superior medial right	51.82 (10.89)	61.21 (19.53)	63.62 (15.86)	4.51	0.02*
	middle left	52.26 (15.56)	65.99 (21.52)	67.04 (17.09)	3.85	0.03*
	middle orbital left	31.69 (10.19)	35.45 (12.85)	33.26 (11.27)	0.32	0.73
	inferior triangularis left	29.01 (6.88)	33.12 (10.34)	31.74 (11.19)	1.19	0.31
	inferior orbital left	32.25 (8.13)	32.15 (8.83)	33.36 (8.63)	0.11	0.90
	medial orbital left	53.09 (13.87)	64.47 (17.94)	56.47 (12.27)	2.33	0.11
Amygdala left		32.18 (7.44)	33.24 (10.58)	33.77 (6.89)	0.05	0.95
Putamen left		82.39 (15.34)	85.86 (11.49)	86.65 (14.43)	0.50	0.61
Rolandic operculum left		46.23 (10.31)	52.68 (15.81)	50.67 (11.48)	1.26	0.29
Precuneus left		94.96 (18.12)	102.07 (19.39)	100.86 (25.79)	0.49	0.62
Precuneus right		103.41 (15.56)	111.78 (20.30)	113.55 (22.63)	1.68	0.20
Precentral left		83.22 (19.45)	95.31 (37.86)	86.29 (23.79)	1.26	0.30

Table 2 legend Table shows mean (SD) clustering coefficient and local efficiency for each group for the 13 respective nodes that were selected for regional analysis. The test-statistic and *p*-value relate to the group comparison. *p*-values are uncorrected for multiple comparisons.

Post hoc analysis of the clustering coefficient and local efficiency showed a similar pattern in each case, with the patient group having a significantly lower clustering coefficient and local efficiency than the control group ($R^2 = 16-23\%$). Whilst unaffected relatives displayed values of clustering coefficient and local efficiency that were intermediate between patients and controls, these did not significantly differ from either (Table 3, Fig. 3). Figure 3 indicates that the variance of the groups differed substantially, introducing the possibility that a small number of individuals were potentially skewing the distributions. Homogeneity of variance was checked for each group prior to analysis. In the case of the right frontal superior medial node this was not met, however removing one individual from the relatives group which was skewing the variances resulted in homogeneity and no change to our results conducted with the whole group. Similarly it did not affect the pairwise comparisons. Furthermore homogeneity was not met for the case-control comparison within the same node. After exclusion of two from the healthy control group to meet the assumption of homogeneity, once again there were no significant changes to our findings.

Table 3 Post hoc analysis of node specific network analysis

		Comparison	Test-statistic	<i>p</i> -value
<u>Clustering coefficient</u>				
Frontal-	superior medial right	rel V con	-0.54	0.59
		pat V con	-3.02	0.005**
		pat V rel	-1.77	0.09
	middle left	rel V con	-0.31	0.76
		pat V con	-2.48	0.018*
		pat V rel	-1.55	0.13
<u>Local efficiency</u>				
Frontal-	superior medial right	rel V con	-0.40	0.69
		pat V con	-2.77	0.009**
		pat V rel	-1.87	0.07
	middle left	rel V con	-0.13	0.89
		pat V con	-2.64	0.012*
		pat V rel	-1.95	0.06

Table 3 legend Comparison shows to which groups the pairwise comparison pertains. con – control, pat – patient and rel – relative groups respectively. *p*-values are uncorrected for multiple comparisons.

Insert Fig. 3 here

Fig. 3 Clustering coefficient and local efficiency metrics in each participant group in key frontal nodes

Fig. 3 legend Scatter plots of node specific analysis with group mean and SEM error bars included. Top line with *p*-value refers to the *p*-value obtained from the group analysis including all three groups. The lower line with *p*-value refers to the *p*-value obtained from pairwise comparison between the bipolar and healthy control group.

3.4 Correlation analysis

No significant correlations were found between the network measures highlighted here and any of the clinical measures investigated; symptom severity scores, age at illness onset and duration of illness.

4. Discussion

Here we used a state of the art technique to examine structural connectivity in bipolar disorder. Global connectivity in the brain appears to be intact in patients with bipolar disorder compared to their healthy family members and unrelated healthy controls. However when we refined our investigation to focus on regions more likely to be involved in the pathophysiology of bipolar disorder, by virtue of prior abnormalities detected in functional imaging assessment of performance during cognitive and emotional tasks, we find evidence supporting impaired frontal connectivity in bipolar disorder.

Differences in local efficiency and clustering coefficient were seen in the left middle frontal gyrus and right medial superior frontal gyrus, with the left medial superior frontal gyrus just failing to reach significance between the groups. These group differences were driven by reduced metrics, clustering coefficients and local efficiency, in the patient group compared to the controls. Despite the small sample and effect sizes the pattern was consistent in each of these regions with the relative group, although not significantly differing from either, consistently lying intermediate between patient and control groups.

We can interpret these metrics in terms of segregation and integration, two properties of small world networks enabling efficient communication both locally and between distant regions (Rubinov and Sporns, 2010; Sporns, 2011). Local efficiency is a measure of how well the node is integrated in the network. High efficiency indicates that the node is connected along relatively direct paths minimising noise and maximising the speed of possible communication between the node and others in the network (Bassett et al., 2009; Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Efficiency is strongly affected by path length while clustering coefficient, related though independent, is based on the frequency of connections between neighbouring nodes and is therefore a measure of segregation (Bullmore and Sporns, 2009). High values indicate highly connected subnetworks. Both segregation and integration are integral to a healthy brain network

(Sporns, 2013). Our findings suggest both impaired integration and segregation in the right medial superior frontal gyrus and the left middle frontal gyrus of patients with bipolar I disorder.

These regions are involved in multiple aspects of high order cognitive processing including emotional regulation and executive function. The medial superior frontal gyrus is involved in self-referential thought (Northoff and Bermpohl, 2004; Northoff et al., 2006) and is part of default mode network (Gusnard and Raichle, 2001).

Abnormalities in these regions have been reported previously in structural and functional MRI investigations of bipolar disorder (Drevets, 2001; Strakowski et al., 2005), and of course in the current cohort hence their selection for regional analysis (Drapier et al., 2008; Surguladze et al., 2010). Interestingly, in a recent study by Marchand and colleagues (2014), similar regions were implicated in a study of functional connectivity in patients with euthymic bipolar II disorder. The authors reported an increase in functional connectivity in the patients compared to controls during a motor activation task in two cortical midline structure (CMS) circuits; medial left SFG - dorsolateral left SFG and medial right SFG - dorsolateral left SFG - medial right FG. These overlap with the regions in which we found structural deficits in segregation and integration. The authors of the former paper conclude that functional CMS circuitry dysfunction continues during euthymia in BP II. Our current findings suggest these circuits are structurally abnormal during euthymia in BP I. Furthermore Leow (2013) and colleagues, who also used network measures, showed deficits in similar areas. Together with previous findings this study adds weight to the hypothesis that the default mode network and areas involved in emotional regulation are regions associated with disrupted connectivity in patients with BP.

Our previous studies of the diffusion data in the same cohort revealed widespread group differences; reduced fractional anisotropy (FA) in the BP group compared to healthy controls as investigated with voxel based analysis (VBA) and targeted diffusion tensor tractography (Chaddock et al., 2009; Emsell et al., 2013). Although these have very different methodologies they

complement each other to investigate focal white matter abnormalities. The current study employed a methodology to investigate the organisation of the brain, this revealed no global differences between groups as measured with network analysis. This may be due to local differences in connectivity, as previously found, being undetectable when investigated globally rather than at a voxel or tract level. It may also be that these local changes may not affect the whole network organisation, as measured by the metrics herein. Furthermore the former studies investigated FA as a measure of white matter organization, either voxel based or averaged over an isolated tract, while the current study used the number of reconstructed connections between regions to infer connectivity strength from which we construct a network to analyse.

We probed our data to investigate the potential of network measures as endophenotypic markers of bipolar disorder. We found that the relatives of bipolar I disorder patients did not differ significantly from either the healthy controls or patient group, failing to support these metrics as potential endophenotypic markers. The effects in these regions were subtle however and the intermediate values for relatives suggested further exploration in a larger sample size would be warranted. However, following a power analysis using effect sizes generated in this study we see that approx. 800 participants per group would be required to show a statistically significant difference between the Control and Relative group, essentially ruling it out as a potentially useful endophenotypic marker. Alternative connectivity measures like 'rich club' may be worth exploring as it has been reported as abnormal in relatives of those with schizophrenia (Collin et al., 2014). Finally, given evidence from other studies that genetically susceptible relatives of patients with bipolar disorder display disrupted structural connectivity as measured by other DTI metrics these may be of more use as endophenotypic markers than those investigated here (Chaddock et al., 2009; Emsell et al., 2013; Skudlarski et al., 2013; Sprooten et al., 2013).

Limitations

This is an exploratory investigation in an area of research that is evolving quickly. It is by no means exhaustive, choosing to focus on a number of the available metrics that have previously been frequently and robustly used in network based analysis. Furthermore there are many methodological options for analyses of this kind, with the optimal choices remaining uncertain for metrics such as node definition, reconstruction of streamlines and weighting of the edges.

We chose the AAL atlas to define our nodes as this has been successfully used previously (Iturria-Medina et al., 2008; Gong et al., 2009; Lo et al., 2010; Caeyenberghs et al., 2012, 2014; Reijmer et al., 2013a, 2013b) and employed the non-tensor based tractography method of constrained spherical deconvolution (CSD) to reconstruct the streamlines (Jeurissen et al., 2011). CSD holds significant advantages over previously used tensor-based models in its ability to estimate multiple contributing fibre orientations within a voxel, making the reconstruction of fibre bundles in areas of crossing fibres possible (Tournier et al., 2007; Jeurissen et al., 2013) and has been successfully used previously in combination with network analysis (Reijmer et al., 2012, 2013a). We weighted the edges by number of streamlines as has previously been successfully done (Bassett et al., 2011; Leow et al., 2013), however caution should be taken in the interpretation of number of streamlines as the number can be artificially enhanced or deflated by numerous factors including crossing fibres, although as noted above this possibility is minimized by the use of CSD. Finally we chose to count streamlines that passed through 2 nodes, whether or not they began/ended in those nodes. This was done so as not to exclude connections between intermediate nodes along streamlines. Despite our careful selection of methods, caution must be taken in relating these findings to other studies using different methodological pipelines. For a discussion on these and other limitations of network analysis see the review by Fornito and colleagues (2013).

Finally, our choice of nodes was driven by prior hypotheses, however we tested 13 nodes without further multiple comparison correction. Given the anatomical relatedness of our regions Bonferonni correction could be considered overly strict. However if we reduced our p-value threshold for group

analysis to 0.01, only the superior medial right node would survive as a significant finding. We presented the results without this correction as our methodology is of considerable interest and our sample size modest, however, we must caution that these findings require replication in a larger sample.

Conclusion

The segregation and integration of whole brain networks of those affected with BP I disorder do not differ from their unaffected relatives or unrelated healthy controls. However at a local level in frontal regions, anatomical connectivity as investigated through network analysis is disrupted in patients. This may suggest structural connectivity dysfunction within the default mode network or emotional regulation areas of patients with BP I disorder.

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Conflict of Interest

None

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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